Research Article

The Association of Dopamine Receptor D2 (*DRD2*) and Vasoactive Intestinal Peptide (*VIP*) Polymorphisms on Egg Production in High Egg Strain of Pradu Hangdum Chiangmai Chickens

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Abstract | The objectives of the research were to determine the association of Dopamine Receptor D2 type (*DRD2*) and Vasoactive Intestinal Peptide (*VIP*) on egg production in 281 high egg strain of Pradu Hangdum Chiangmai chickens (HPHC). PCR-RFLP protocols were used to identify polymorphisms of both genes. The frequencies of TT, TC and CC genotypes of *DRD2* were 0.05, 0.40, and 0.55, respectively. The frequencies of T and C alleles of *DRD2* were 0.25 and 0.75, respectively. Regarding *VIP*, the frequencies of II, ID and DD genotypes were 0.59, 0.28, and 0.13, respectively. The frequencies of I and D alleles of *VIP* were 0.73 and 0.27, respectively. The association of *DRD2* polymorphism were detected on egg number at 360 days (EN360) and egg per month (EM) ($P<0.05$). The chickens carrying the CC genotype had higher EN360 and EM (189.82 eggs and 15.81 eggs) and the ones carrying the TC (180.98 eggs and 15.08 eggs) and the TT genotype (176.38 eggs and 14.69 eggs) was non-significant. The *VIP* polymorphism were associated on egg number at 270 days (EN270), EN360 and EM ($P<0.01$). The DD genotype had higher EN270, EN360 and EM when compared to the ID and II genotypes. The *DRD2* and *VIP* genes were moderate polymorphism and their alleles could be the potential genetic markers for chickens' selection to improve egg number.

Keywords | Gene marker, Native chickens, Egg production, *DRD2*, *VIP*

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INTRODUCTION

Native chickens are important for rural households as a source of high-quality protein. The advantage characters are the ability to tolerate the harsh environmental condition and resistant to diseases (Padhi, 2016). Moreover, meat of native chicken has lower contents of fat and cholesterol (Jaturasitha *et al*., 2016; Bungsrisawat *et al*., 2018). Thai native and Thai native crossbred chicken showed high anserine anserine/carnosine antioxidant substances carcass and meat quality yields (Charoensin *et al*., 2021). Eggs are considered rich sources of protein and iron (Kusum *et al*., 2018). High egg strain of Pradu Hangdum Chiangmai chickens (HPHC) are a native chicken that the breeding objective is to increase egg production by joint cooperation between the Department of Livestock Development and the Agricultural Research Development agency (Public organization). Pradu Hangdum is one of four native chickens in Thailand, which maintain the flock in Chiang Mai Livestock Research and Breeding Center, the researchers aimed to improve egg production for produce more chick for the farmers. HPHC was developed from Pradu Hangdum Chiangmai 1, with meat quality and appearance characteristics that are preferred by consumers (Kammongkun *et al*., 2015). Therefore, the objective of breeding program was to increase 30% egg production from 147 egg/year of foundation stock to 191 egg/year of breeding stock. The characteristics of Pradu Hangdum

chickens are that they have a feather color of black, a small pea comb, color of face is red and whitish yellow skin (Kammongkun *et al*., 2015). Nowadays, consumers have an increasing demand for native chicken because of native chickens are generally raised without using antibiotics or chemicals, and thus are rendered safe and have no negative impact on human health (Funaro *et al*., 2014).

The limitations of native chickens are low egg production and slow growth. If the hens give higher egg production, the costs of chicks and costs of fattening will reduce. Therefore, genetic improvement has been made to improve the native chickens to increase egg production and growth performance. The selected chickens can growth better than non-selection in open housing (Promket and Ruangwittayanusorn, 2021). However, for egg production there are many ways to improve. The identification of polymorphism and DNA marker associated with egg production traits could be used as marker assisted selection (MAS) which lead to genetics improvement to increase egg production of native chickens.

In recent years, the molecular technologies and genetic marker approach have gradually become the mainstream for genetic improvement in breeding program. Furthermore, molecular breeding can significantly improve breeding efficiency and shorten the breeding period, so using molecular marker breeding has an advantage in breeding. Egg production has importance economic traits in poultry that are complex quantitative traits involving many genes and their interactions. Many researches focus on gene marker associated with egg production that can be used to improve egg production (Liu *et al*., 2019; Syed *et al*., 2019; Zhuang *et al*., 2019; Tenzin *et al*., 2020). From the research results, it was found that using molecular markers to selecting high egg production in chickens was success. Therefore, a molecular marker that influenced egg production in HPHC population was a good decision for genetic improvement to increase egg productions.

Dopamine, an abundant neurotransmitter in the central nervous system and periphery, has been shown to play important roles in cognition, emotion, endocrine function, and hyperprolactinemia in mammals (Xu *et al*., 2010a). Dopamine has five dopamine receptors subtypes (*DRD1-DRD5*) have been classically divided into two classes referred to as D1-like (*DRD1* and *DRD5*) and D2-like (*DRD2*, *DRD3*, and *DRD4*) receptors based on their pharmacological, biochemical, and physiological differences (Xu *et al*., 2010b). In avian, dopamine, played a critical role in prolactin secretion and stimulating prolactin secretion via dopamine receptors D1 at the hypothalamic level and inhibiting prolactin secretion via dopamine receptors D2 (*DRD2*) at the pituitary by operating through vasoactive intestinal peptide (Xu *et al*., 2010a). Prolactin is a negative regulator of avian reproductive activity such as incubating behavior. Inhibit pituitary secretion of prolactin by *DRD2,* inhibit incubation activities and to improve egg production in chickens.

Vasoactive intestinal peptide (*VIP*) controls prolactin hormone by binds to specific receptors on the lactotroph cells in the anterior pituitary. Moreover, the protein level of VIP and gene expression correlate with circulating prolactin levels during the different reproductive stages and stimulate the secretion of prolactin and regulate prolactin (Zhou *et al*., 2010). Vasoactive intestinal peptide immunoneutralization reduces prolactin gene expression and prolactin secretion induced by electrical stimulation of the hypothalamus (Halawani *et al*., 2000). Association studies between variation of *VIP* gene and egg production traits have been carried out in poultry and found that five polymorphisms were associated with the total number of eggs in chickens (Zhou *et al*., 2010; Xu *et al*., 2011b; Ngu *et al*., 2015). Since there was no the information of *DRD2* and *VIP* genes on egg production in Thai Native Chicken. Therefore, it would be required to study the association between gene markers on egg production will be benefit for genetic improvement in native chicken. The objective of this research was to identify the association of *DRD2* and *VIP* genes on egg production traits in HPHC.

MATERIALS AND METHODS

Ethical consideration

Institution Animal Care and Use Committee (IACUC) of Mahasarakham University, Mahasarakham, Thailand, approved the use of animals under this study (IACUC-MSU-007/2020).

Animals and egg production record

In this study, a total of 281 at the age of sixteen weeks of HPHC were randomly selection under the open house at Chiangmai Livestock Research and Breeding Center, Sanpatong District, Chiangmai Province, Thailand. The chickens were provided feed and water *ad libitum* using commercial diet (17% CP and 2,900 kcal of ME/kg for laying period) (NRC, 1994). At 16 weeks of age, they were reared in separate cages (8×16 inch). The individual data recording were composted with hen weight at first egg (HWFE), age at first egg (AFE), egg weight at first egg (EWFE), egg weight at 270 days (EW270), egg weight at 360 days (EW360), egg number at 270 days (EN270), egg number at 360 days (EN360) and egg per month (EM).

Blood collection and DNA extraction

Blood samples (1 mL) were collected from the wing vein into a tube containing 100 µL of 0.5 M

ethylenediaminetetraacetic acid (EDTA). This was used as an anticoagulant.

Genomic DNA was isolated from whole blood using the guanidine hydrochloride method (Goodwin *et al*., 2011). Briefly, cell lysis buffer and protein precipitation buffer were added to the blood. Cell lysate was then centrifuged for 5 minutes at 10,000 rpm at 4°C. The supernatant was then transferred to 1.5 mL tube, and absolute isopropanol was added. The DNA was precipitated at 10,000 rpm for 5 minutes at 4°C. The supernatant was discarded, and DNA pellet was washed 2 times with 75% ethanol. The DNA pellet was air-dried at room temperature and dissolved in DNA hydration buffer. The DNA quality and concentration were determined by Nanodrop 2000c spectrophotometer (Thermo Scientific, USA). The DNA was diluted to 50 ng/µL as a working solution and stored at –20°C before use.

PCR amplification

The reactions of PCR mixture were carried out in a total volume of 10 µL containing: 1 µL of diluted genomic DNA (50 ng/µL), 1 µL of 10X PCR buffer, 0.8 µL of 50 mM $MgCl_2$, 1 µL of 1 mM dNTPs, 1 µL of 5 µM of each primer, add 0.1 µL of *Taq* DNA polymerase (Promega, San Diego, CA) and 4.1 µL of nuclease free water. PCR amplification was carried out in a PCR thermal cycle (COBETT RECHEARCH, Australia 2003, iCycler thermal cycler, BioLad, U.S.A) conducted under the following conditions: Pre-heating at 94°C for 5 minutes followed by 35 cycles at denature 94°C, 30 s; annealing temperature (Table 1), 40 s; and extension 72°C, 30 s. Final extension was carried out at 72°C for 5 minutes and the amplified products were hold at 4°C until needed. The PCR product of *DRD2* and *VIP* genes were analyzed using 2% agarose gel. After electrophoresis at 100 V for 35 minutes, gel was stained with GELSTAR™ (Gelstar Inc, NY) for 10 minutes. DNA fragments were visualized by gel documentation (Lab Focus, Inc.).

Restriction fragment length polymorphism (RFLP) analysis

Polymerase chain reaction (PCR) products from each chicken were digested separately with 1 restriction enzymes (*Bse*GI for *DRD2* and *Vsp*I for *VIP*). Each digestion reaction contained 2 µL of PCR products, 1 µL of cut smart, and 0.2 µL of the restriction enzyme, add deionized water 6.8 µL in a total volume of 10 µL. Subsequently, each reaction was incubated overnight at 55°C for *Bse*GI and 37°C for *Vsp*I. After digestion, the products were visualized by 2.5% agarose gel electrophoresis for 40 minutes, at 100 V, and the genotypes were determined with Gel Documentation (Lab Focus, Inc.) by GELSTAR™ (Gelstar Inc, NY) staining.

Statistical analysis

The means of egg production traits were analyzed by PROC MEANS (SAS Institute Inc. Cary, NC, 2003). Allele and genotype frequencies, polymorphism information content (PIC) and the Hardy-Weinberg equilibrium (HWE) were tested based on Chi-square test ($\chi 2$) according with Falconer and Mackay (1996).

The associations between the genotypes of the candidate genes (*DRD2* and *VIP*) and diplotype on egg production were analyzed using the following model:

$$Y_{ij} = \mu + G_i + e_{ij}$$

Where; Y_{ij} is phenotypic record (egg production traits) in the chickens, μ is the overall population mean, G_i is the fixed effect of the genotype (*DRD2* or *VIP* or diplotype) and e_{ij} is the residual error.

RESULTS AND DISCUSSION

Egg production in high egg strain of Pradu Hangdum Chiangmai chickens (HPHC)

The means (Means), standard deviation (SD), minimum (Min) and maximum (Max) of egg production traits in HPHC were presented in Table 2. Means of HWFE and EWFE were 1,928.99 g and 33.80 g, respectively. The AFE was found at 153.89 days, cumulative of egg number at 270 days (EN270) and 360 days (EN360) were 150.34 and 185.70 eggs, respectively. Moreover, the EW270 and EW360 were 44.71 g and 44.80 g, respectively.

Genotypes and allele frequencies of *DRD2* and *VIP* genes in HPHC

Two candidate genes (*DRD2* and *VIP*) were identified from HPHC. The PCR product of *DRD2* and *VIP* were

Table 1: Sequences of primer and annealing temperatures (Ta) for PCR amplification.

Genes	Location (bp)	Chr. [3]	Gene ID	Primer sequence (5′-3′)	Length [4] (bp)	Ta [5] (°C)	Enzyme
DRD2 [1]	T5841629C	Chr.24	428252	F: tgcacataaaagcccactcactg	248	60	*Bse*GI
				R: gcctgagctggtgggggg			
VIP [2]	AGG Indel	Chr.3	396323	F: gaaacccatctcagtcatccta	306	58	*Vsp*I
	D2648-2650I			R: accacctatttttccttttctac			

Note: [1] Ngu *et al*. (2015); Xu *et al*. (2011a; b); [2] Vu and Ngu (2016); [3] Chr. is chromosome [4] Length is the length of PCR Products; [5] Ta is annealing temperature.

Table 2: Means, standard deviations (SD), minimum (min) and maximum (max) values for egg production parameters.

Traits	Means	SD	Min	Max
HWFE (g)	1,928.99	193.02	1,075.00	2,482.00
AFE (day)	153.89	11.24	130.00	198.00
EWFE (g)	33.80	5.63	21.20	55.00
EW270 (g)	44.71	3.05	32.48	54.12
EW360 (g)	44.80	3.07	32.28	54.36
EN270 (egg)	150.34	32.72	53.00	221.00
EN360 (egg)	185.70	30.85	83.00	266.00
EM (egg)	15.47	2.57	6.92	22.17

Note: HWFE is hen weight at first egg, AFE is age at first egg, EWFE is egg weight at first egg, EW270 is egg weight at 270 days, EW360 is egg weight at 360 days, EN270 is egg number at 270 days, EN360 is egg number at 360 days and EM, egg per month.

248 bp and 306 bp (Figures 1, 2), respectively. The single nucleotide polymorphism (SNP) of *DRD2* was transition mutations one nucleotide (T>C or C>T), whereas *VIP* was INDEL variations based on the addition or removal of AGG nucleotides (D2648-2650I). The PCR products were digested with *Bse*GI for *DRD2* and *Vsp*I for *VIP*. The RFLP pattern of three genotypes on *DRD2* were genotype TT (196 bp and 52 bp), genotype TC (248 bp, 196 bp and 52 bp) and genotype CC (248 bp). While digestion with *Vsp*I of *VIP* were genotype II (306 bp), genotype ID (306 bp, 154 bp and 152 bp) and genotype DD (154 bp and 152 bp).

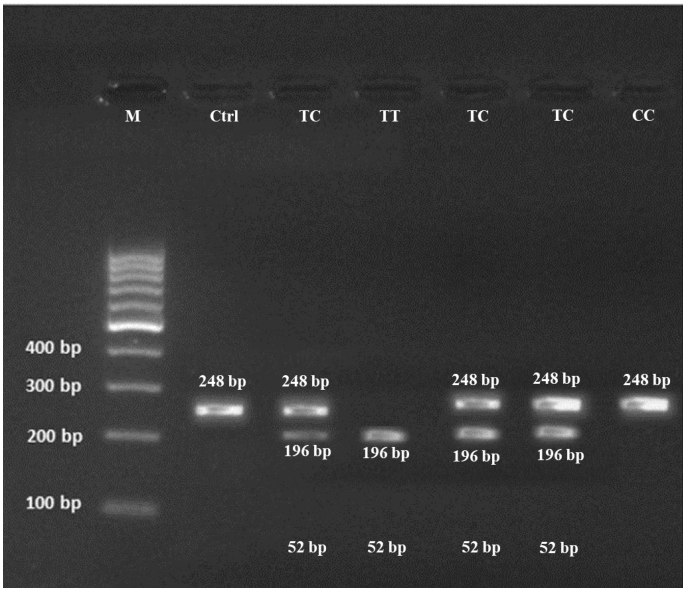

Figure 1: The genotype of *DRD2* gene: M = marker 100 bp, Ctrl = PCR product (248 bp), genotype TT (196 bp and 52 bp), genotype TC (248 bp, 196 bp and 52 bp) and genotype CC (248 bp) digested with *Bse*GI.

The distribution of the genotype and allele frequencies are shown in Table 3. Three genotypes of *DRD2* gene (TT, TC and CC) were found with frequencies ranging from 0.05 to 0.55 and allele T (0.25) lower frequency than allele C (0.75). The allele frequencies of the *VIP* genes were observed for allele I (0.73) and allele D (0.27). For the *VIP*, the highest genotype frequency was II pattern (0.59) and genotype frequency for ID and DD were 0.28 and 0.13, respectively (Table 3). The *DRD2* and *VIP* are followed HWE and fitted the assumption of the equilibrium. The average polymorphism information content (PIC) value of *DRD2* and *VIP* genes were 0.31 and 0.32, respectively. Results showed that the *DRD2* and *VIP* were moderate polymorphic in HPHC.

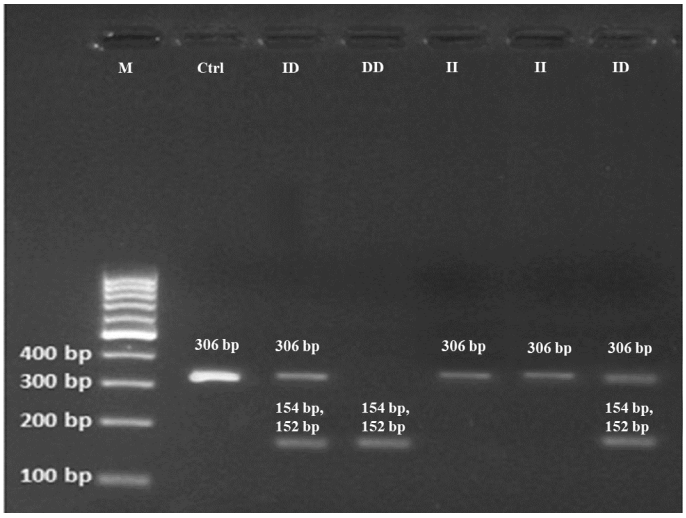

Figure 2: The genotype of *VIP* gene: M = marker 100 bp, Ctrl = PCR product, genotype II (306 bp), genotype ID (306 bp, 154 bp and 152 bp) and genotype DD (154 bp and 152 bp) digested with *Vsp*I.

Table 3: Genotype and allele frequencies of polymorphisms

Gene	Total	Genotype frequency			Allele frequency		$\chi 2$	PIC
		TT	TC	CC	T	C		
DRD2	281	0.05 (13)	0.40 (111)	0.55 (157)	0.25	0.75	0.08	0.306
		II	ID	DD	I	D		
VIP	281	0.59 (166)	0.28 (79)	0.13 (36)	0.73	0.27	0.12	0.321

Note: PIC is polymorphism information content; $\chi 2$ (1, 0.05) = 3.84.

Association of polymorphisms in the *DRD2* and *VIP* genes with egg production

Association of the polymorphisms in the two candidate genes with egg production in HPHC were analyzed and the results were shown in Table 4. The significant effects of *DRD2* polymorphism were detected on EN360 and EM ($P<0.05$). The chickens carrying the CC genotype had a great of EN360 and EM than the ones carrying the TC and TT genotype. However, chicken with TC genotypes had EN360 and EM values were not different from TT

genotypes. The CC genotype of *DRD2* had high EN360 (189.82 eggs) than the TC and TT genotype (180.98 and 176.38 eggs, respectively). Moreover, the CC genotype gave EM was 15.81 eggs, which was greater than the TC and TT genotype, which gave the EM were 15.08 and 14.69 egg, respectively.

Table 4: Association between genotype of *DRD2* and *VIP* gene on egg production.

Gene	Traits	Genotype			P-value
		TT	TC	CC	
DRD2	HWFE (g)	1964.77	1930.14	1925.04	0.77
	AFE (day)	156.46	153.74	153.78	0.70
	EWFE (g)	33.00	34.44	33.41	0.29
	EW270 (g)	45.08	44.68	44.70	0.90
	EW360 (g)	45.07	44.88	44.72	0.87
	EN270 (egg)	150.76	151.08	149.79	0.94
	EN360 (egg)	176.38 ^{b}	180.98 ^{b}	189.82 ^{a}	0.03
	EM (egg)	14.69 ^{b}	15.08 ^{b}	15.81 ^{a}	0.03
VIP		II	ID	DD	P-value
	HWFE (g)	1940.69	1903.89	1930.17	0.39
	AFE (day)	153.10	155.43	154.13	0.28
	EWFE (g)	33.81	33.65	34.04	0.88
	EW270 (g)	44.95	44.30	44.51	0.28
	EW360 (g)	45.04	44.41	44.56	0.28
	EN270 (egg)	145.74 ^{B}	151.21 ^{B}	169.66 ^{A}	0.003
	EN360 (egg)	181.88 ^{B}	185.26 ^{B}	204.33 ^{A}	0.005
	EM (egg)	15.15 ^{B}	15.43 ^{B}	17.02 ^{A}	0.005

Note: ^{ab} means within a row with different superscripts different significant (P<0.05). ^{AB} means within a row with different superscripts different significant (P<0.01). HWFE is hen weight at first egg, AFE is age at first egg, EWFE is egg weight at first egg, EW270 is egg weight at 270 days, EW360 is egg weight at 360 days, EN270 is egg number at 270 days, EN360 is egg number at 360 days and EM, egg per month.

The highly significant association was found between the *VIP* and EN270, EN360 and EM (p<0.01). The DD genotype had higher EN270 (169.66 eggs) and EN360 (204.33 eggs) value compared to the ID (151.21 and 185.26 eggs) and the II genotypes (145.74 and 181.88 eggs). The association of *VIP* gene was found in EM. Chicken with DD genotype showed higher EM than ID and II genotype (P<0.01).

Association between the diplotypes of *DRD2* and *VIP* gene on egg productions

The *DRD2* and *VIP* genes were the candidate genes that effected on egg production traits. Egg production traits are control by multiple genes. Therefore, the combine genotype between the *DRD2* and *VIP* genes on egg production was studied. A total of 281 HPHC with 9 diplotypes were obtained based on these three genotypes. Five diplotypes with frequencies greater than 5.0% were analyzed for association with egg production. The CC-II diplotype has the highest frequency with 33.50%. The result showed the significant association between diplotype and EN270, EN360 and EM (P<0.01). The CC-DD diplotype had the highest EN360 (206.30 eggs) and EM (17.19 eggs). The others diplotype were non-significantly different among them (Table 5).

The egg production of HPHC in this study which similarly with previous study of Worawit *et al.* (1998) reported mean of weight at first egg of hen was 1,900 g. Moreover, Tongsiri *et al.* (2019) reported mean of body weight at first egg in Thai native chickens was 2.05 kg and egg weight at first egg was 36.94 g. The average annual egg productions of Thai native chicken (Pradu Hangdum) were 117±41 eggs (Mookprom *et al.*, 2017). Previous studies found that candidate gene such as *DRD2* and *VIP* genes had effect on egg productions in native chickens (Xu *et al.*, 2011a; Ngu *et al.*, 2015). The size of the DNA fragment from the RFLP technique in this study as same the reported by Ngu *et al.* (2015).Most alleles frequency was similar to those previously reported in Chinese native chickens (Ningdu Sanhuang) and Vietnam chickens (Noi chickens) (Xu *et al.*, 2011a; Ngu *et al.*, 2015). The PIC value is often used to measure the polymorphism of allele fragments. According to Ding *et al.* (2010), PIC>0.50 indicates a highly informative

Table 5: Association of the diplotype in *DRD2* and *VIP* gene with egg productions.

Diplo type	n	Egg productions							
		HWFE (g)	AFE (day)	EWFE (g)	EW270 (g)	EW360 (g)	EN270 (egg)	EN360 (egg)	EM (egg)
CC-DD	23	1934.00	154.47	33.84	45.06	45.01	71.01 ^{A}	206.30 ^{A}	17.19 ^{A}
CC-ID	40	1876.20	156.42	33.04	44.13	44.21	152.37 ^{B}	190.87 ^{B}	15.90 ^{B}
CC-II	94	1943.94	152.48	33.45	44.85	44.87	143.50 ^{B}	185.34 ^{B}	15.44 ^{B}
TC-ID	37	1940.22	154.18	34.52	44.50	44.67	150.43 ^{B}	180.10 ^{B}	15.00 ^{B}
TC-II	62	1922.39	153.83	34.46	45.00	45.20	149.06 ^{B}	177.93 ^{B}	14.82 ^{B}
P-value		0.44	0.46	0.66	0.63	0.62	0.007	0.001	0.001

Note: HWFE is hen weight at first egg, AFE is age at first egg, EWFE is egg weight at first egg, EW270 is egg weight at 270 days, EW360 is egg weight at 360 days, EN270 is egg number at 270 days, EN360 is egg number at 360 days and EM, egg per month. ^{AB} means within a row with different superscripts different significant (P<0.01).

locus, 0.25<PIC<0.50 indicates a reasonably informative locus, and PIC<0.25 indicates a slightly informative locus. In HPHC population, *DRD2* and *VIP* were reasonably informative loci. In this study, allele C for *DRD2* gene and allele I for *VIP* gene were high proportion than allele T and D, respectively. According with Xu *et al.* (2010a, 2011b) report that frequency of C allele was higher than T allele in *DRD2* gene. In addition, *VIP* gene also showed higher proportion of I allele (Ngu *et al.*, 2015). In HPHC, allele and genotype frequencies of *DRD2* and *VIP* follow the Hardy-Weinberg law due to these HPHC were G_0 flock and selection for egg production traits. These were recruited from 800 chicks and selected by 2-month cumulative egg production with a low selection intensity and using only phenotype selection. As a result, both genes were not affected by selection and were in HWE. From Kubota *et al.* (2019) explain that HWE was influenced by many factors, including selection, the rate of recombination and mutation, genetic drift, the mating system, population structure, and genetic linkage.

In the present study, we detected a significant difference between genotype and EN360 and EM for *DRD2* gene. In a similar study, Ngu *et al.* (2015) showed the significant associations between genotype of *DRD2* and *VIP* gene on egg numbers in indigenous Noi chickens. Moreover, Ningdu Sanhuang population detected polymorphisms associated of *DRD2* gene with egg number at 300 days of age, chickens with TT genotype were higher egg number at 300 days compared to that of the CC and TC genotypes (Xu *et al.*, 2011b). Tenzin *et al.* (2020) found in Pradu Hangdam chickens that DRD2 was associated with egg weight at first egg, with TC and TT genotypes having higher breeding values than the CC genotype.

Moreover, we found that *VIP* genotype associated with EN270, EN360 and EM. Also, another study showed the 5' region of *VIP* gene was related to egg production (Zhou *et al.*, 2010). On the other hand, Xu *et al.* (2011b) proved that the *VIP* gene was not significant associated with egg number at 300 days of age in chickens. The dopaminergic system plays an important role in the regulation of the avian reproductive system (Xu *et al.*, 2010b). Vasoactive intestinal peptide is prolactin releasing factor in chicken, stimulates and regulates the secretion of prolactin hormone, that is an important role in incubation behavior in chickens (Zhou *et al.*, 2010). Furthermore, dopamine was demonstrated to play a dual role in prolactin release by affecting *VIP* secretion, exerting its inhibitory effect via dopamine D2 receptor (*DRD2*).

Results of diplotype analysis also showed that it was associated with EN270, EN360 and EM. In the same way, Xu *et al.* (2011a) found that the seven diplotype were significant associated with age at first egg traits. The result of this study showed that the polymorphism of *DRD2* and *VIP* gene which associated with egg number. Thus, CC genotype in the *DRD2* gene, as well as DD genotype of the *VIP* gene, may be important genotype for high egg number in chickens.

CONCLUSIONS AND RECOMMENDATIONS

DRD2 and *VIP* were analyzed using the PCR-RFLP technique for detection of genotypes. This study found the *DRD2* polymorphism was associated with EN360 and EM, while *VIP* polymorphism was associated with EN270 EN360 and EM. Chickens with CC and DD genotype have better egg number in High Egg Strain of Pradu Hangdum Chiangmai chickens (HPHC). Therefore, these genes could be used as genetic marker for egg number to HPHC chicken breeding program.

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NOVELTY STATEMENT

This is the first report to study the association of DRD2 and VIP genes in High Egg Strain of Pradu Hangdum Chiangmai pupulation.

AUTHOR'S CONTRIBUTION

DP: Study conception and design, analysis and interpretation of results, draft manuscript preparation, reviewed the results and approved the final version of the manuscript.
KP: Collected egg production data and chicken blood samples, DNA extraction and genotype analysis
KR: Analysis and interpretation of results, approved the final version of the manuscript.
JK: Collected egg production data and chicken blood samples, approved the final version of the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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